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CONTENTS

Figures

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Embryonic Development of the Drywood Termite, *Cryptotermes brevis*[1]

C. Y. KAWANISHI

INTRODUCTION

The 2000 species of termites belonging to the order Isoptera are phylogenetically related to the cockroaches (Matheson, 1951; McKittrick, 1965). The order is comprised of six families, five extant and one fossil (Weesner, 1960). Despite their interesting phylogeny and economic importance, their embryology has been virtually ignored. Detailed studies of the embryogenesis of only five species have been made to date. Knower (1900) noted the "strikingly orthopteran" development of *Eutermes* sp. (probably *ripperti).* Strindberg (1913) studied the embryogenesis of *Eutermes rotundiceps* and found its embryonic disc similar to that of the apterygote *Lepsima* because both were minute in relation to the size of the egg. Toth (1943) and Geigy and Striebel (1959) published short studies on the embryology of *Kalotermes falvicollis.* Subsequently, a detailed description of *K. flavicollis* embryology and an accompanying study of *Zootermopsis nevadensis* (Hagen) were published (Striebel, 1960). From these studies it was concluded that the termite embryo was of the extreme short type like that of Orthoptera. However, the termites differed because their embryos maintained a superficial position to the yolk and segmentation progressed caudally from the preantennal segment. The embryogenesis of *Odontotermes redemanni* Wasmann (Mukerji and Chowdhuri, 1960) also differed from those of other insects because the amnion formed as a specialized tail fold of the embryonic disc and was many cells thick from the time of its formation. Additionally, the serosa had a double mode of origin: the portion covering the embryo originated from the tail fold, while the remainder was derived from the blastoderm of that region. The endomesoderm formed by tangential division of germinal epithelium cells and not from a gastrular invagination.

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[1]This paper is a thesis submitted to the Graduate School of the University of Hawaii in partial fulfillment of the requirements for the Master of Science degree.

The five species of termites whose embryogenesis has been studied belong to families virtually on opposite ends of the phylogenetic scale of Isoptera as presented by Weesner (1960). *K. flavicollis* and *Z. nevadensis* belong to the relatively primitive Kalotermitidae and Hodotermitidae, respectively. *O. redemanni, Eutermes (ripperti?),* and *E. rotundiceps* are, in contrast, from the most highly evolved family, Termitidae.

The subject of this study, the drywood termite *Cryptotermes brevis* (Walker), is a kalotermitid of considerable economic importance. Relatively little is known about its life history and behavior. Studies on this termite have been concerned mostly with methods of control, but colony development and feeding relationships were treated in work published by McMahan (1962, 1963). Colonies usually do not contain more than 300 individuals and live entirely within galleries formed by their feeding. This species, like other members of the family, has no true worker caste and the work is done by nymphal forms of the soldiers and reproductives. *C. brevis* was chosen for the embryological study partly for contrast with the more highly evolved species of Termitidae, and partly for the practical reason that eggs of known ages are easily obtained. Only the major events in the embryogenesis of *C. brevis* were studied in detail. They were: (1) cleavage and formation of the blastoderm, (2) development of the embryonic disc and differentiation of the embryonic envelopes, (3) formation of the inner layer, (4) growth and segmentation of the embryo, (5) blastokinesis, (6) formation and degeneration of the secondary dorsal organ, and (7) dorsal closure and differentiation of the mesoderm.

MATERIALS AND METHODS

Previous investigations (McMahan, 1962) with *C. brevis* indicated that supplementary reproductives oviposit more frequently than do primary ones in incipient colonies. Supplementary forms, identifiable by their darker pigmentation and nymphal characteristics, developed in about 3 weeks when groups of large nymphs were isolated from functioning reproductives. Eggs for this study were obtained from supplementary reproductives.

Groups of 15 large nymphs were isolated in termitaries constructed of a length of birch wood tongue-depressor sandwiched between two glass microscope slides. Termites were placed in a rectangular 1 x 3 cm hole centrally located in the wood. The bottom slide was taped to the depressor at the ends and the top slide clamped on with bent paper clips. Eggs were collected by removing the clips and laterally displacing the top slide. Daily collections of eggs, obtained with a moist artist's paint brush, were placed in 35 mm x 7 mm specimen vials containing a layer of termite fecal pellets. The presence of fecal pellets improved the survival of the eggs. Collection continued until

the first few batches of eggs hatched, and the incubation times of the individuals were recorded. The mean of the daily temperature was 28 C and ranged from 23 to 31 C.

The remaining eggs, which ranged in age from one to 56 days, were used in the embryological study. Eggs an even number of days old (2, 4, . . . 56) were fixed in Kahle's fixative. Eggs an odd number of days old were fixed by the same procedure the following day. Each age category contained 15 to 20 eggs and thus the study was based on about 450 eggs. Care was taken to keep eggs of different ages separated during fixation so that the chronology of major embryonic events would be preserved.

To insure rapid penetration the eggs were pricked with fine dissecting needles while immersed in the fixative. This was done in an embryological watch glass under a dissecting microscope. After 24 hours fixation the eggs were removed, washed in 70 percent ethanol for 24 to 48 hours, and then stored in fresh 70 percent ethanol. Fixation increased the opaqueness of the embryo so that it contrasted sharply against the translucent yolk. Because some features became too transparent to study after clearing and staining, many of the gross observations were done at this time. Malformed embryos were discarded.

Due to normal variation and incubation at an ambient room temperature some embryos deviated in developmental rate, which necessitated determination of an average state of development for each category. The developmental stage exhibited by the majority of embryos within the age category was termed "average", and deviants were assigned to other age categories by comparison with average specimens. Most deviants were placed in an age category on either side of the one in which they were present. Characteristics used to identify different age categories were: length of the embryo in relation to length of the egg; number of segments present, and presence or absence of various flexures, curvatures, and structures.

Whole mounts were prepared by removing specimens from 70 percent ethanol, staining them overnight in Grenacher's borax carmine, and differentiating them in acidified 70 percent alcohol. Embryos were washed in several changes of 70 percent ethanol and dehydrated in 95 percent and absolute ethanol. Clearing was done by transferring the embryos through a graded series of absolute alcohol and xylene or clove oil. Xylene caused severe distortion of younger eggs but gave good clear specimens with older embryos. Oil of cloves cleared to a lesser extent but gave undistorted specimens of all stages—this was advantageous in studying external changes. Embryos were mounted in Canada balsam along with glass capillaries to prevent the cover slip from squashing the specimens.

When eggs were to be sectioned, chorions were removed with fine dissecting needles. During dehydration in ethanol, embryos were stained with

either eosin or triosin to facilitate visualization during the subsequent embedding process. Clearing was done in xylene and specimens were embedded in Paraplast®. Gradual infiltration was accomplished by hardening a volume of Paraplast in a vial at room temperature, introducing the embryo in about 1/5 that volume of clearing agent, and heating the vial to 56 C. Two changes of pure Paraplast at 30 and 60 minutes each followed, after which the embryo and some Paraplast were picked up with a flame-warmed medicine dropper and placed on a microscope slide. As the Paraplast hardened and thickened, the embryo was quickly oriented with a flame-warmed needle while being viewed with a dissecting microscope. The edges of the glass slide were used as references during orientation. A sectioning block was then attached, again using the edge of the slide as a guide to retain orientation. The slide was gently warmed over a flame, and as the Paraplast melted on the bottom the attached specimen and block were pulled off.

Specimens were sectioned with a rotary microtome at 7 to 9 microns. Sections were attached to slides with egg albumin. The Paraplast was removed from sections with xylene and rehydrated in a descending alcohol series for staining with Delafield's or Heidenhain's hematoxylin. Sections were again dehydrated, cleared with xylene, and mounted in Canada balsam. Younger embryos stained better with Delafield's hematoxylin, while Heidenhain's proved more satisfactory with older specimens.

Live embryos were studied in an aqueous medium with the aid of a dissecting microscope. Addition of small amounts of wetting agent to the water rendered the chorion transparent. Between observations the eggs were dried on filter paper.

All drawings were made with the aid of a camera lucida and are somewhat diagrammatic, but the various organs and structures are properly oriented.

DEFINITION OF TERMS

The anterior and posterior poles or ends of the egg are those in which the head and tail, respectively, of the embryo will lie when the embryo is in its definitive or final position. By contrast, the head and tail of the embryo itself, unless referred to as such, will be designated "cephalic" and "caudal," respectively. The ventral and dorsal sides of the embryo and egg of *C. brevis* remain the same throughout embryonic development except during rotation, so no distinction need be made.

The bilaterally symmetrical termite egg can be assigned three axes: an anterior-posterior one, the longitudinal axis; a dorsal-ventral (dorso-ventral) axis; and a left-right axis. When reference is made to rotation around a specific axis, it means that the rotary motion will, if extended for 360

degrees, describe a more or less circular plane perpendicular to the axis. Other terminology used to denote spatial relationships follows the definitions of Steyskal (1945).

When the embryo first differentiates from the extraembryonic regions it will be referred to as the "embryonic anlage", as distinct from the "germ band", which represents the stage in development when primary germ layers are established (after Counce, 1961). The term "embryonic disc" will be used in a broader sense in reference to the shape of the embryo at these early stages.

The formation of the germ layers in insects and other animals having yolk-rich eggs are indistinct processes. What constitutes gastrulation, if it indeed occurs, is still a much debated question. An inner layer is formed and produces not only endoderm (as expected) but also mesoderm. In a strict sense the term "endomesoderm" should be used, as the inner layer secondarily produces some endoderm.

In this study, tissues have been designated as mesoderm and endoderm according to the structures or parts to which they give rise. Johannsen and Butt (1941) cite Spemann (1938) as saying that homologizing is possible only after the formation of the anlagen, i.e., at the developmental period when the individual parts of the germ band have become differentiated, if not in their outwards appearance at least in their developmental tendency. Tiegs and Murray (1938) also state that, "Homology of an anlage is determined by its fate rather than its origin . . . ". Therefore the tissue that forms the definitive midgut epithelium is designated as endoderm in this study. Similarly, the tissue forming somatic musculature, fat cells, splanchnic musculature, etc., is referred to as mesoderm.

ABBREVIATIONS

The following abbreviations are used in figures 2 through 9.

A	Anterior
Abd.	Abdominal segment
Am.	Amnion
Amc.	Amniotic cavity
Amd.	Amniotic depression
Amf.	Amnioserosal fold
Ant.	Antenna
Bw.	Body wall
Caud.	Caudal curvature
Cav.	Cavity
Ceph. Hyp.	Cephalic hypodermis
Ceph.	Cephalic lobes
Ch.	Chorion
Chr.	Chromatin clump
Coel.	Coelomic cavity

Coel. Sac	Coelomic sac
Col.	Columnar cells
Cons.	Constriction
Cren.	Crenations
D	Dorsal surface
Deut.	Deutocerebrum
D. Cl.	Dividing cells
Ect.	Ectoderm
Emb. Ar.	Original embryonic area
Emb. D.	Embryonic disc
End.	Endoderm
Eps.	Epineural sinus
Fc.	Fat cells
Gang.	Ganglion
Hd.	Head
Hemc.	Hemocoel
Ht.	Heart
Inl.	Inner layer
Lft	Left side
Lr.	Labrum
Mand.	Mandibular segment
Max.	Maxillary segment
Mdg.	Midgut
Mes.	Mesoderm
Mns.	Median nerve strand
Mp.	Medial mesodermal proliferation
Ms.	Median mesodermal strand
Musc.	Muscle
Nuc.	Nucleus
P	Posterior
Pleur.	Pleuropodia
Pren.	Preantennary segment
Procm.	Protocorm
Procph.	Protocephalon
Reg. Pr.	Region of proliferation
Rt	Right side
SDO	Secondary dorsal organ
Ser.	Serosa
Sernl.	Serosal nucleus
S. Mes.	Somatic mesoderm
S. Mus.	Somatic muscle
Sp.	Space
Spl. Mes.	Splanchnic mesoderm
Stom.	Stomodaeum
Tel.	Telson
Th.	Thoracic segments

Tri.	Tritocerebral segment
V	Ventral surface
Vac.	Vacuoles
Vism.	Visceral musculature
Vitm.	Vitelline membrane
Vitel.	Vitellophags
Y.	Yolk
Yg.	Yolk granule
Y. Syn. M.	Yolk syncitial membrane
Zg.	Zone of growth

RESULTS

Incubation period

Figure 1 shows the distribution of incubation times of 32 *C. brevis* eggs held at a mean temperature of 27.5 C. The mean incubation time was 56.0± 3.6 days. There was one deviant with an incubation period of 68 days.

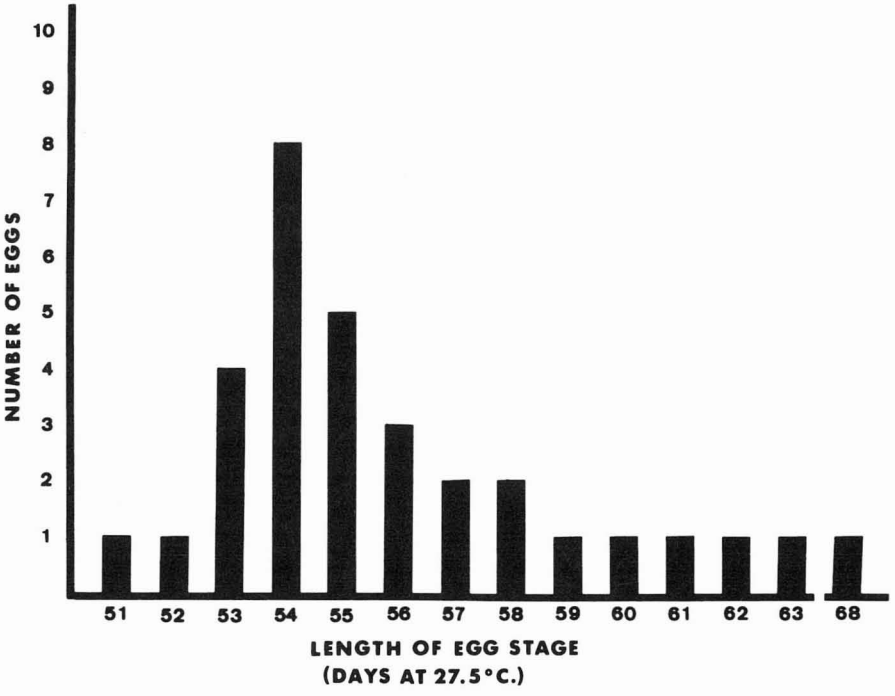

FIG. 1. Histogram of incubation periods exhibited by sample of *C. brevis* eggs.

Description of the egg

Eggs of *C. brevis* appeared to be laid indiscriminately throughout the termitaries by the supplementary reproductives. Newly-laid eggs were identifiable because they adhered to the surfaces on which they were deposited. As the eggs dried they detached and were scattered among the fecal pellets in the termitaries.

C. brevis eggs were elongate and slightly curved. They were bilaterally symmetrical and rounded at the poles. Their mean length was 1.13 ± 0.02 mm and the mean width at the midpoint was 0.50 ± 0.01 mm. The widest point was in the posterior half at about 57 percent of the egg length. The eggs were convex dorsally and slightly concave ventrally.

The young eggs were pinkish, turgid with yolk, and relatively translucent. As the embryo developed the eggs turned progressively opaque and whitish.

The chorion of the newly-laid egg was resilient and translucent. Externally it was sculptured with minute, roughly hexagonal facets forming a network over the entire egg. The chorion remained taut until prior to hatching, at which time it more loosely enclosed the fully-developed termite and appeared dry and wrinkled. A short medio-dorsal suture was present on the chorion near the anterior pole. At eclosion the termite emerges through an opening formed along this line.

The transparent vitelline membrane, present immediately beneath the chorion, delimited egg contents prior to formation of the blastoderm. A distinct cytoplasmic reticulum was observed when stained, but oosomes, micropyles and periplasm were not observed.

Cleavage and formation of the blastoderm

Eggs with two cleavage nuclei were the youngest stages obtained. These nuclei were surrounded by elongate protoplasm which was pentagonal in profile with strands trailing out from the corners. In eggs having only two nuclei, one nucleus was always nearer the posterior end and the other slightly antero-dorsal (Fig. 2A); this indicated that syngamy and subsequent first cleavage division occurred near the posterior pole. The four nuclei resulting from the second cleavage division were located slightly anterior to the position previously occupied by their respective mother nuclei (Fig. 2B), with one of the four still nearer the posterior pole. After three cleavage divisions, the nuclei were still in the posterior half of the egg (Fig. 2C).

Mitosis became asynchronous after either the fourth or fifth cleavage division. Of the 16 nuclei shown in Figure 2D undergoing the fifth cleavage, two are in interphase, one in prophase, four in metaphase, one in anaphase, and eight in telophase. Clearly, if this division were synchronous all the nuclei would be in the same phase of mitosis or nearly so. At this stage the

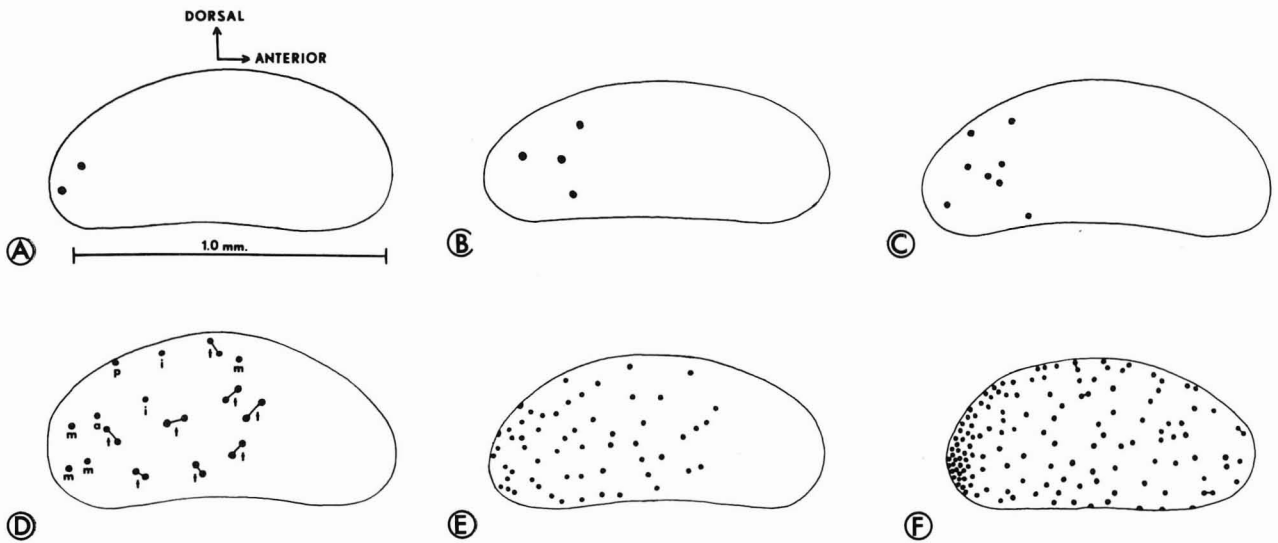

FIG. 2. Position of nuclei in eggs of *C. brevis* at various stages during cleavage. **A.** 2-nuclei stage. **B.** 4-nuclei stage. **C.** 8-nuclei stage. **D.** 16-nuclei stage. Note asynchrony of this (5th) cleavage division. Letters designate phases of mitosis: i = interphase; p = prophase; m = metaphase; a = anaphase; t = telophase. **E.** 59-nuclei stage. Some nuclei in the posterior pole have reached dorsal surface. **F.** Blastoderm stage.

nuclei had passed the midpoint of the egg, and some were in the extreme posterior region of the egg but none was at the surface.

By the 59-nuclei stage (Fig. 2E) the nuclei in the posterior pole had reached the yolk surface and were interconnected by protoplasmic strands. These nuclei were more densely clustered than were nuclei elsewhere. Enmeshed in the protoplasmic strands of the nuclei in the extreme posterior region were small spherical bodies, usually in pairs or triplets; these stained darkly with borax carmine and were easily visible at 100x magnification. The anterior-most nuclei, located about two-thirds of the distance to the anterior pole, were in metaphase while all others at the surface were in interphase.

By the next stage (Fig. 2F) most of the nuclei were at the surface and formed a layer of cells, the blastoderm. Those remaining in the yolk were vitellophags or yolk cells. Fewer nuclei were present in the anterior than in the posterior pole.

Formation of the embryonic disc and envelopes

By the fourth day of embryonic life the cytoplasms of the blastoderm cells were clearly delineated. Such cells were proliferating at the yolk surface (Fig. 3A) in the apical and dorso-medial areas of the posterior pole. Cells were also present just below the surface at the extreme posterior pole; these cells were distinguishable from vitellophags because the latter are larger, amoeboid in appearance, and possess more granular nuclei.

A decrease in the number of cells in the anterior half of the blastoderm (Fig. 3B) was noted concurrently with an increase of cells in the posterior proliferating regions. During differentiation of the embryonic disc from the blastoderm, the nuclei in the actively proliferating disc region seemingly congregated more closely and formed a more compact embryonic anlage. Sections of such discs showed that they were already several cells thick.

At about the time the nuclei started to form the blastoderm, a morphogenetic movement occurred at the posterior end of the egg (Figs. 3C, D, E). The movement was a retraction of the yolk to form a small cavity into which the embryo gradually grew (Fig. 3F).

The incipient amniotic depression appeared as a concavity of the central portion of the embryonic disc on about the fifth day. The disc (Fig. 4A) lay more or less superficially on the yolk and was continuous with the extra-embryonic remnants of the blastoderm, the serosa. The serosa, the outermost of the two embryonic envelopes, eventually enclosed all of the egg. The second envelope, the amnion, formed later and covered only the ventral surface of the embryo.

As the amniotic depression deepened, the posterior edge of the embryonic disc curled anterior-dorsally (Fig. 4B) and retracted from the yolk. The

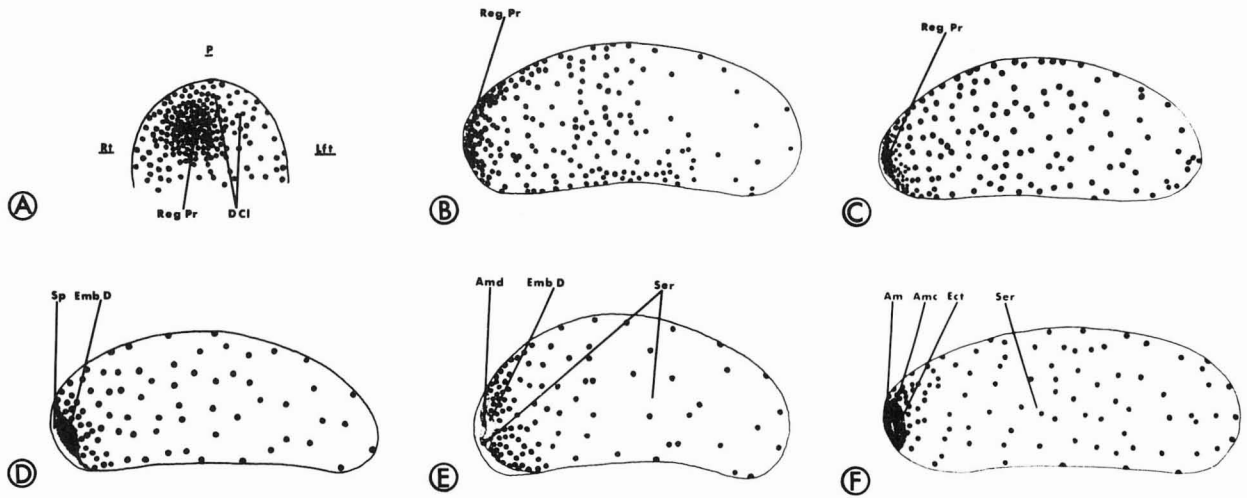

FIG. 3. Proliferation of cells in posterior pole of egg and formation of young embryo. **A.** Posterior polar view of egg showing nuclei aggregation and division in presumptive embryonic region. **B.** Side view of posterior pole at a slightly later stage showing increase in number of cells. **C.** Rudiments of embryonic disc. **D.** Embryonic disc with incipient amniotic depression. **E.** Embryonic disc with upturned posterior margin marking beginning of amnioserosal fold. **F.** Embryo with amniotic cavity and multilayered amnion.

adjoining serosa then pulled away, creating a small space devoid of yolk. An apparent yolk syncytial membrane underlying the entire embryonic disc prevented the yolk from entering the cavity. Longitudinal sections revealed proliferation of the amnioserosal fold from the inner edge of the posterior rim of the concave disc (Fig. 4C). The fold grew over the depression and fused with the cells on the lateral and anterior rims of the embryonic disc to form the amniotic cavity on about the eighth day. In succeeding days the now spherical vesicular embryo elongated and the amnion thinned out into a monolayer (Fig. 4D).

The inner layer (endomesoderm)

During the period of progressive deepening of the amniotic depression, between the fourth and sixth days and prior to the formation of the amnioserosal fold, cells on the under side of the depression near the center (Fig. 4B) submerged and multiplied by tangential division to form the endomesoderm or inner layer. The oval cells of the inner layer contrasted with the columnar ones of the ectoderm, and the inner layer appeared thickest near the center of the disc.

Subsequent to elongation and ventral growth via the posterior pole, a monolayer of endomesoderm underlay most of the embryo. The endomesodermal cells in the region of the presumptive prothorax were flattened, in contrast to the oval ones at or near the growing tips of the embryo. The inner layer nuclei were oriented approximately perpendicular to those of the ectoderm.

Sagittal and transverse sections of 14- to 18-day-old embryos showed mitotic division of inner layer cells cephalad to the caudal apex. Numerous mitotic figures were not observed at the apex proper. This indicated perhaps that some inner layer cells constantly formed *de novo* from the under side of the embryo during the elongation process. Histologically, cells of the amnion and the inner layer were contiguous and appeared to have a common origin in the growing zone at the lateral and caudo-lateral junction of the amnion and the embryonic band (Fig. 8A). Possibly, original cells of the inner layer came from the under side of the ectoderm but secondary cells originating at the junction reinforced the inner layer. Such movement of cells may be due to either active migration or mechanical displacement. More detailed studies are needed to elucidate the mechanism of formation of the inner layer and to determine the origin of such cells.

Growth and segmentation

The elongation of the *C. brevis* embryo followed the approximate curvature of the egg. Starting from the posterio-dorsal region of the egg the embryo grew around the curvature of the posterior pole onto the ventral

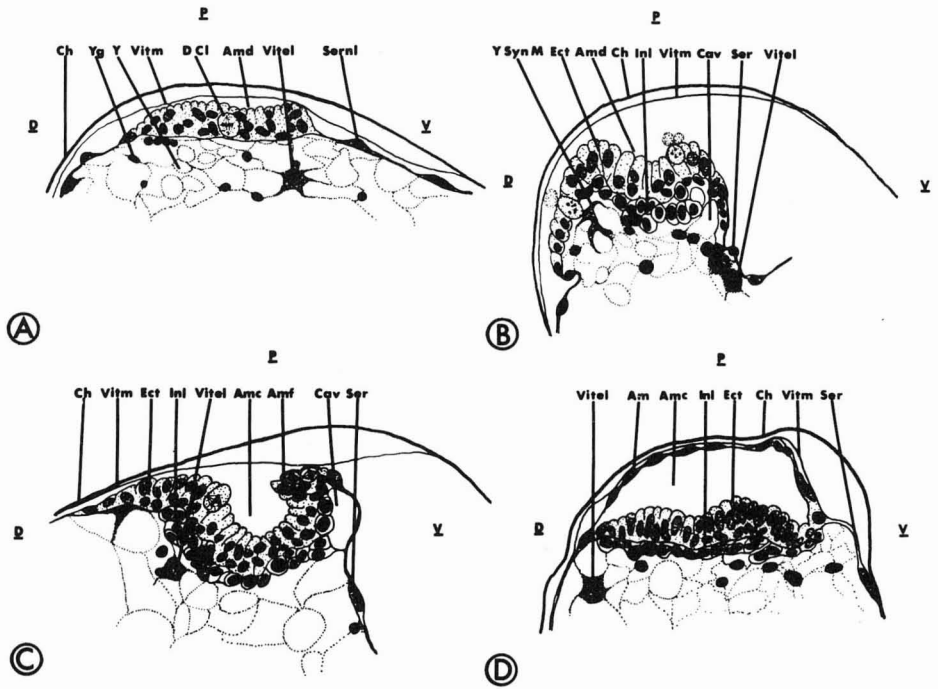

FIG. 4. Longitudinal sections of posterior pole of *C. brevis* eggs. **A.** Early embryonic disc. **B.** Embryonic disc at stage comparable to that in Figure 3D. Note presence of inner layer and slight concavity within the disc. **C.** Embryonic disc with rudimentary amnioserosal fold. Mitotic figures are present in disc and fold area. This stage is comparable to that of Figure 3E. **D.** Slightly elongated embryo with enclosed amniotic cavity.

surface of the egg. Growth continued until the cauda of the embryo nearly reached the anterior pole of the egg. The embryo grew slightly diagonal to the medial plane of the egg and maintained a position superficial to the yolk. The amnion became increasingly membranous during elongation.

Gross examination of the embryo between the 10th and 12th day revealed growth of the caudal perimeter of the embryonic vesicle (Fig. 5A). The boundary of the original vesicle was still apparent at this time. The embryonic vesicle occupied the entire apical region of the posterior pole of the egg and from a lateral view was roughly C-shaped.

By the 14th day the embryo had acquired cephalic lobes and was divisible into a protocephalic or primary head region and a protocormic or primary trunk region (Fig. 5B). Ventrally, the embryo exhibited indications of segmentation and formation of the anterior oral segments. Sections of embryos of this age showed complete segmentation of the inner layer and partial segmentation of the ectoderm in the region of the future oral and thoracic segments. An amorphous mass of cells which represented the zone of growth was present at the caudal apex.

Sixteen days after oviposition the embryo was about 3/4 the length of the egg (Fig. 5C). Distinguishable within the protocephalon were the following segments: (1) preantennary with large lateral lobes; (2) the deutocerebral, with a pair of caudally-growing protuberances representing the future antennae; and (3) tritocerebral or inter-calary, which do not bear any appendages in this insect. Medially behind the preantennary segment were the raised bilobed rudiments of the labrum. The incipient stomodaeum, a circular invagination, was present caudal to and partially between the lobes of the labrum.

The protocorm consisted of six distinct segments, each possessing lateral rudiments of future appendages. The three cephalic segments, the mandibular and the first and second maxillary, composed the gnathal segments. The second maxillary fused later to form the labium. The three thoracic segments were also identifiable.

The antennae of 18-day-old embryos were longer and the oral and thoracic appendages more distinct (Fig. 5D). Six or seven abdominal segments were present, and the more cephalad ones possessed evanescent embryonic appendages called pleuropodia. At this age the labrum was a flat rectangular structure that overlapped the stomadael invagination. The embryonic tail was bent inward towards the yolk in the area of the fifth abdominal segment. The region between the presumptive 6th and penultimate abdominal segments formed a U-shaped flexure into the yolk. The last section of the abdomen, the telson, was larger than the preceding segments and bore a median invagination that was the rudiment of the proctodaeum. The segments appeared to develop from the large tail.

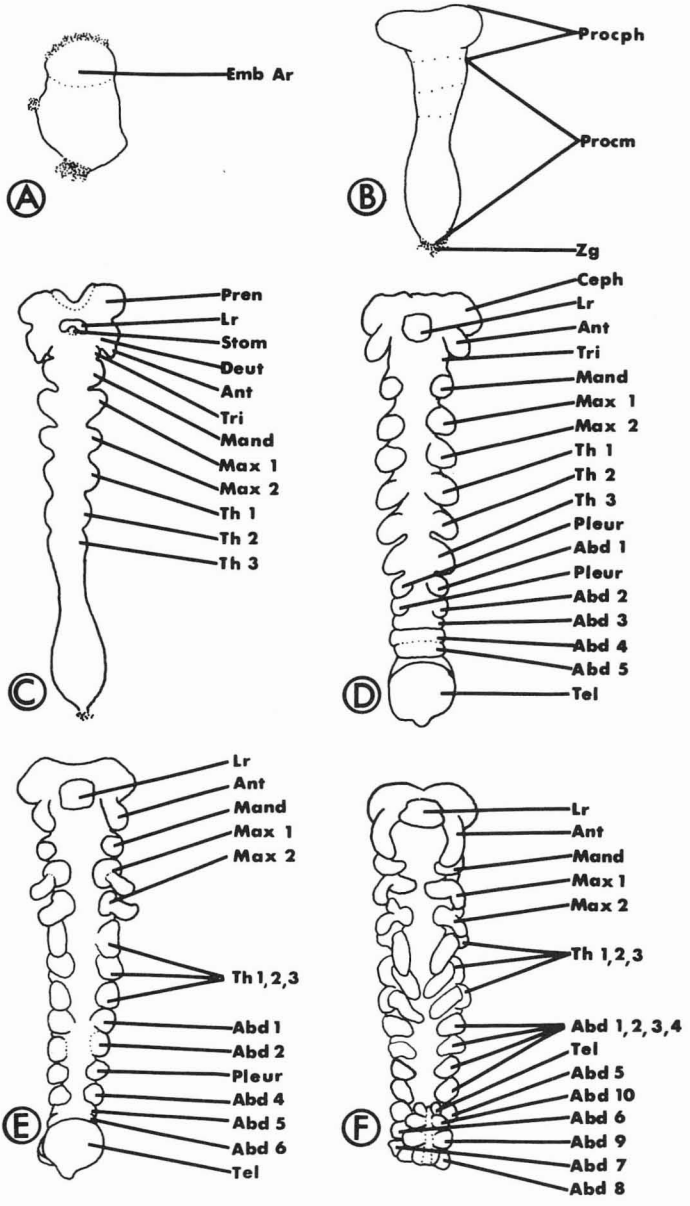

FIG. 5. Stages in growth and segmentation of *C. brevis* embryo. Embryo in Figure 5A is slightly older than that in Figure 3F.

At 20 days a few additional abdominal segments appeared and the labrum became noticeably larger (Fig. 5E). The mesal borders of the basal portion of the first and second maxillary segments commenced growth towards each other. The distal region of the mandibular appendages curve medially, while those of the maxillary segments curve laterad.

The gnathal segments all possessed bilobed appendages (Fig. 5F) by the 22nd day. All ten abdominal segments and the much reduced telson were present. This seemingly marked the end of segmentation.

Pleuropodia were present on all segments except the telson. Those on the first abdominal segment possessed narrow tubular structures distally. The flexure between the 6th and 7th abdominal segments was no longer apparent; instead, the cauda curved cephalad in the region of the 7th and 8th abdominal segments. The elongated thoracic legs now projected caudally.

Blastokinesis

The embryo of *C. brevis* exhibited two blastokinetic movements. The first was a ventral displacement of the elongating embryo commencing about the 12th day. Previously, the C-shaped embryo was oval and occupied the apical curvature of the posterior pole. In ensuing days the embryo elongated and concurrently the whole germ band was ventrally displaced. By the 14th day all but the cephalic region of the embryo was on the ventral side of the egg. The protocephalon was still bent slightly dorsally and was located in the posterior pole. On the 16th day the slightly longer embryo was completely ventral in position. This marked the end of the first blastokinetic movement of the embryo.

The second blastokinetic movement was a complex rotation associated with the emergence of the embryo from within the embryonic envelopes. Impending embryo rotation was signaled between the 28th and 30th days by flexure of the cephalic lobes backwards until the face of the embryo was perpendicular to the ventral surface of the egg. The embryo at this stage appeared short, thick, and S-shaped because of the flexure and the caudal curvature. The yolk diminished slightly and the apical region of the posterior pole was empty (Fig. 6A). The embryonic envelopes, fused together adjacent to the labrum, were pulled taut against the face of the embryo. An opening through which the embryo commenced to exit developed in the region of the fusion of the two envelopes, but the edges of two envelopes surrounding the orifice remained united. The initial movement of the embryo was dorso-anteriorly around the left-right axis of the egg. When the embryonic head reached the dorsal surface and moved anteriorly a short distance, rotation appeared to commence around the second axis (Fig. 6B). This sideways rotation was directed ventrally around the longitudinal axis of the egg. At this time the second thoracic segment was partially out of the enve-

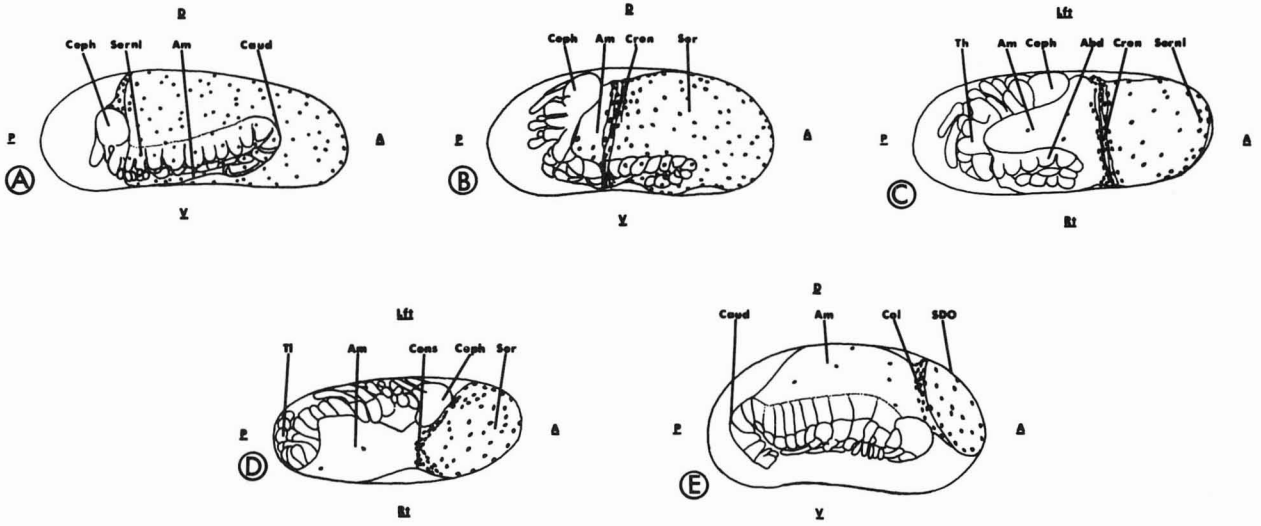

FIG. 6. Embryo undergoing rotation. **A.** Left side view of cephalically and caudally flexed embryo emerging from embryonic envelopes, which illustrates beginning of rotation around left-right axis. **B.** Left side view of embryo commencing rotation around second axis (longitudinal axis of egg). Crenations of serosa adjacent to coalescence with amnion is apparent. **C.** Dorsal view of U-shaped embryo. **D.** Dorsal view of lateral facing embryo with flexed cauda in posterior pole. Crenations in serosa are no longer apparent. **E.** Embryo in definitive position. Embryo has completed rotation and secondary dorsal organ occupies anterior-dorsal region of egg.

lopes and the anterior edges of the cephalic lobes were dorsal in position and anteriorly directed. The meson of the embryo at this time was tilted a few degrees laterad from the meson of the egg.

When rotation started, the serosa enclosed the bulk of the yolk occupying the anterior half of the egg. But as the embryo slipped out of the embryonic envelopes the yolk was gradually displaced into a space created between the dorsal aspect of the embryo and the amnion. Concurrently, the serosa contracted with the diminishing yolk volume. The line of coalescence between the serosa and amnion was easily located at this time because the large serosal nuclei were easily seen with a dissecting microscope.

By the time the embryo had rotated around the shorter axis of the egg to a point where its head lay directly opposite the cauda on the left surface of the egg (Fig. 6C), rotation had proceeded about 90 degrees around the longitudinal axis of the egg. The embryo was U-shaped, with its meson approximately perpendicular to that of the egg. The U-shaped embryo was bent in the thoracic region with the appendages protruding into the posterior pole. The region of union of the serosa and amnion was marked by a constriction girdled by a crenulate band about 2/3 the distance to the anterior pole.

The embryo moved anteriorly with the contracting serosa and in the process the cauda of the embryo was displaced around the posterior curvature to the opposite surface of the egg. When the embryonic head was about 3/4 the distance to the anterior pole and located ventro-laterally, the flexed tail occupied the posterior pole of the egg (Fig. 6D). The end of rotation was marked by the attainment of the definitive ventral position (Fig. 6E). The result of this intricate rotation was the return of the embryo to the ventral surface of the egg but with its head and tail in poles opposite from which they started. The embryo maintained a position superficial to the yolk during rotation. Caudal curvature was retained.

Formation and degeneration of the secondary dorsal organ

The secondary dorsal organ of *C. brevis* formed from the contracted serosa. At the time the embryo emerged from the hole in the region of envelope fusion, the serosa occupied the anterior half of the egg and enclosed about 75 percent of the yolk (Fig. 6A). As emergence continued, the yolk from the serosa was displaced into the space within the amnion formerly occupied by the embryo proper. Crenations of the serosa appeared in the region adjacent to the line of coalescence of the two embryonic envelopes (Fig. 6B and C). The crenations appeared concurrently with the decrease in yolk volume and seemingly resulted from condensation of the serosa. Later, the crenations disappeared with the formation of a constriction in that region. The crenulate area differentiated into a densely nucleated narrow

band girdling the yolk with progressive constriction. This band was located near the midpoint of the egg and was attached (Fig. 6D) ventrally to the embryonic head. A gradual narrowing of the space between the head and the band occurred simultaneously with the anterio-ventral rotation of the embryo.

Subsequent to katatrepsis (Fig. 7A) the diminished sac-like serosa occupied the anterior-most portion of the egg and provisional dorsal closure was effected by the amnion. The band and the contracted sac-like serosa are cumulatively referred to as the secondary dorsal organ. This structure is located over the head of the embryo and becomes apparent on about the 31st day of embryonic life. Sections of the band (Fig. 7D) demonstrated it to be composed of columnar cells contiguous with the membranous serosa anteriorly and the amnion and cephalic hypodermis posteriorly. The nuclei of the band were large, like those of the serosa, and whole mounts of embryos indicated a simultaneous decrease in the size of the sac-like serosa and an increase in the width of the band. These observations indicate that shrinkage of the serosa is due to aggregations into a densely nucleated band of columnar cells.

By 34 to 36 days after oviposition, the secondary dorsal organ was ovoid, apically pointed, and more post-cephalic in position (Fig. 7B). It consisted only of columnar cells having neither lumen nor yolk (Fig. 7E). Many cells appeared to be degenerating, and vacuoles containing oval clumps of dark-staining material were present. The nuclei no longer stained darkly and the nuclear envelope was indistinct. The amnion in regions adjoining the secondary dorsal organ was thicker and more densely populated with nuclei. The boundary between the amnion and secondary dorsal organ was quite distinct because of difference in size of the nuclei.

In succeeding days the secondary dorsal organ rapidly diminished in size. By the 37th day the only remnant of the secondary dorsal organ was an oval disc surrounded by a membranous area located post-cephalically on the dorsal surface of the prothoracic segment (Fig. 7C). Sections of this area (Fig. 7F) revealed masses of dark-staining granular material and indistinct nuclei. The dorsal aspect of this degenerating mass still protruded beyond the encroaching body wall. Subsequently during dorsal closure by the integument, the remnants of the secondary dorsal organ submerged into the body cavity. The last vestiges of the secondary dorsal organ then appeared to disintegrate into the yolk.

Differentiation of mesoderm, midgut formation, and dorsal closure

Subsequent to formation of the endomesoderm on about the 5th day, the mesoderm remained relatively unchanged in appearance. It proliferated into a one-cell-thick layer with the nuclei oriented more or less perpendic-

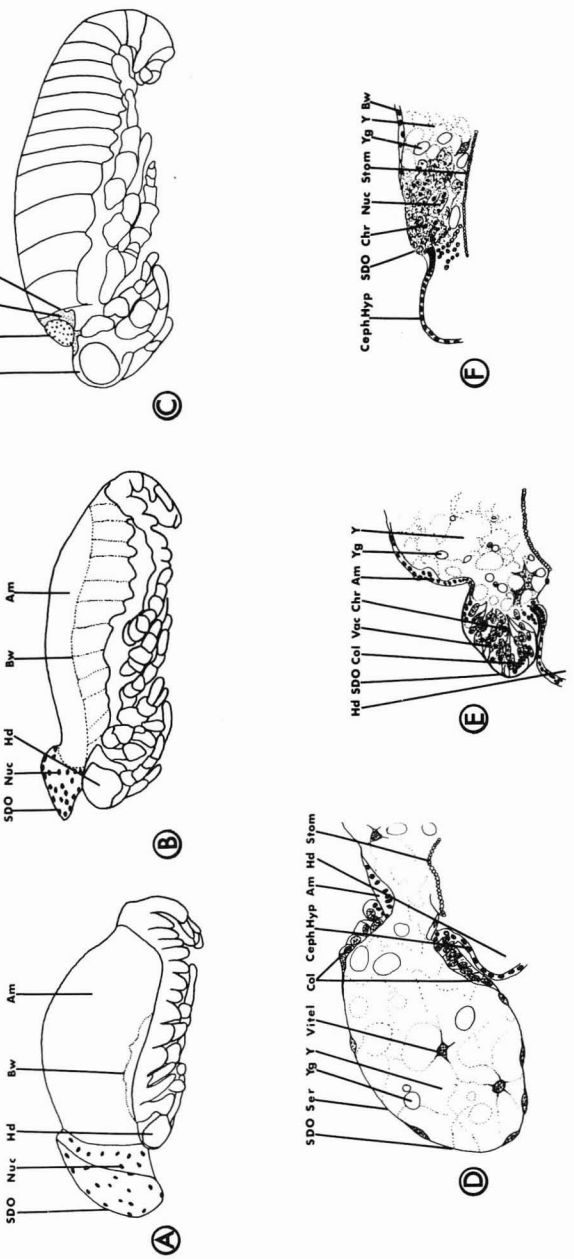

FIG. 7. Stages during degeneration of secondary dorsal organ of *C. brevis* embryo. **A-C.** Side views of secondary dorsal organ in whole mounts of embryos; **D-F.** Longitudinal sections of secondary dorsal organ at stages comparable to those in A-C.

ularly to those of the overlying ectoderm (Fig. 8A). The layer extended nearly the entire length of the elongating embryo, but in the extreme cephalic region was sparse or missing.

Segmentation of the mesoderm was contemporaneous with the external differentiation of the gnathal and thoracic segments. Considerable thinning of the intersegmental and thickening of the segmental regions occurred. The mesodermal segments also thin out medially but not to the extent of the intersegmental areas.

Pairs of lateral lumen in the thoracic and gnathal segments, the coelomic cavities, appeared by the 18th day. The mesodermal tissue surrounding the cavities are called coelomic sacs. The cross section shown in Figure 8B is through a gnathal segment of an 18-day-old embryo. The coelomic cavities in this region are oval, while the caudal-most cavities were either semi-circular or triangular. Mesally-curved monolayer extensions of mesodermal tissue overlapping the developing ventral nerve cord were present on the dorso-medial corner of all coelomic sacs. These extensions were irregular near the midline of the embryo.

The 22-day-old embryo possessed neural grooves and vertically oriented columns of cells (Fig. 8C). At the bottom of each column was a large cell presumed to be the neuroblast. These structures together constitute the median nerve strand. Embryos of this age possessed enlarged coelomic cavities with thinned-out sac lining. In the gnathal segments, coelomic sac enlargements caused by proliferation of the medio-ventral mesodermal cells accompany growth of the appendages. The dorso-medial extension of the mesoderm is no longer apparent. The yolk in the gnathal segments receded from the mesoderm laterally to form the rudiments of bilateral epineural sinuses.

A cross section through a mesothoracic segments of a 26-day-old embryo (Fig. 8D) disclosed changes which occurred in the thoracic and gnathal regions. Ganglia developed in the segments, the yolk receded further from epineural areas in the gnathal somites, and lateral epineural sinuses coalesced medially to form a single definitive epineural sinus. Rudiments of the sinuses were also present at this stage in the thoracic and anterior abdominal segments. In the gnathal and thoracic segments there was further proliferation of the mesoderm, especially in the medio-ventral and medial areas of the coelomic sacs.

Those regions of the coelomic sac mesoderm that were to form the somatic musculature, the fat cells, and the splanchnic musculature (Fig. 8E) were apparent at about the time of cephalic flexure (ca. 28 days). The lateral mesodermal region and its medio-ventral proliferation developed into the somatic musculature, the medial proliferation into fat cells, and the dorsal wall of the sacs into splanchnic musculature. The anlage of the somatic

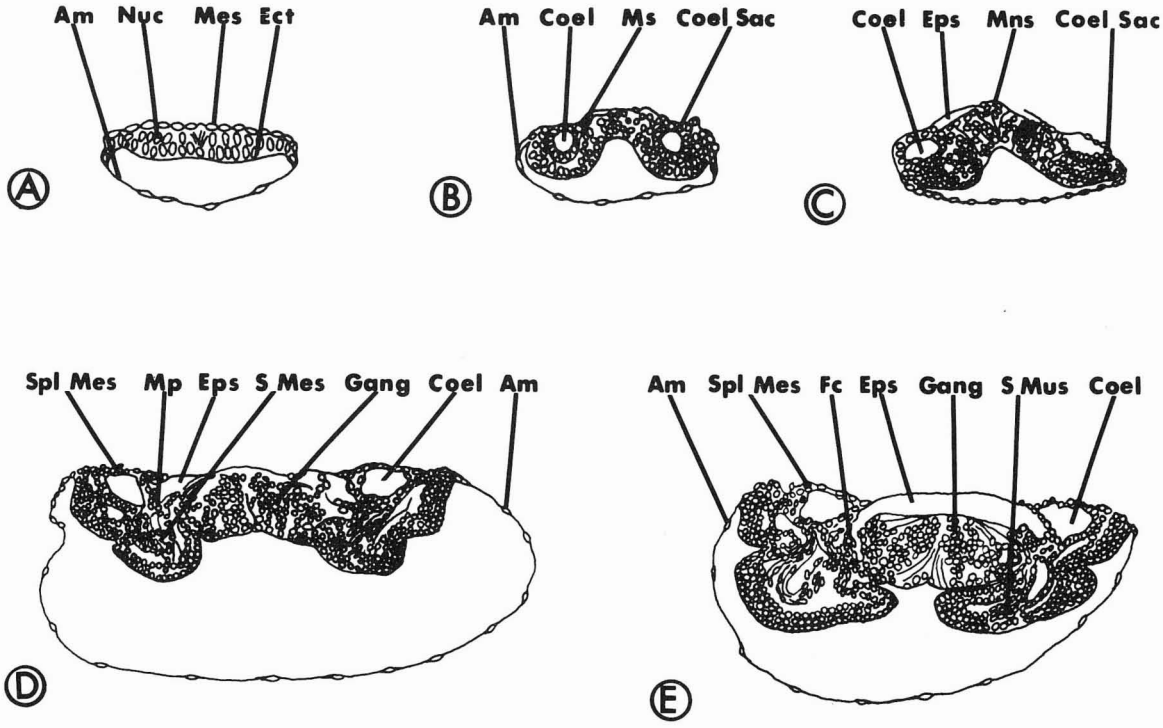

FIG. 8. Stages in differentiation of mesoderm. **A.** 14-day-old embryo. **B.** 18-day-old embryo. **C.** 22-day-old embryo. **D.** 26-day-old embryo. **E.** 28-day-old embryo.

musculature was also developing. Definite epineural sinuses were present in all but the extreme caudal segments.

During rotation the endoderm, which is destined to form the midgut, became evident between the yolk and embryo proper as a pair of ventro-lateral monolayer ribbons of cells extending from the stomodael to the proctodael invaginations. Concurrently the body walls commenced dorsal growth around the yolk and amnion. By the 32nd day of embryonic life the walls and the endoderm have enclosed nearly a third of the ventral region of the yolk mass (Fig. 9A). Dorsally, the yolk is retained by the amnion—ventro-laterally by the paired endodermal ribbons and the contiguous splanchnic mesoderm, and ventrally by an apparent yolk membrane. The inner surface of the ectoderm is lined with somatic mesoderm on either side of the ventral nerve cord to within proximity of the dorsal limits of the body wall. The medial mesodermal walls of the coelomic sacs have begun to differentiate into fat cells. The endodermal ribbons are attached laterally to the splanchnic mesoderm. A membrane seemingly continuous with the endoderm and similar to that present medially between the ribbons, became apparent between the yolk and the dorsal growing edge of the ectoderm. Vitellophags were attached to this membrane.

Nearly two-thirds of the yolk mass was enclosed by the body wall by about the 35th day (Fig. 9B). At this time ventral fusion of the paired endodermal ribbons was imminent, and the lateral somatic mesoderm had differentiated into muscle tissue. The fat cells, which were contiguous laterally with the somatic muscles, formed a conspicuous network with stellate nuclei from the coelomic cavity to the sides of the nerve cord.

The body walls meet dorso-medially (Fig. 9C) on about the 38th day. At this stage the yolk cells stained only lightly and seemed to be degenerating. The cardioblasts (along with the accompanying mesoderm) which were located at the dorsal junction of the splanchnic and somatic mesoderms, have also fused dorso-medially to form the tubular heart. The endoderm has surrounded the yolk to complete definitive dorsal closure. The heart commenced pulsation on about the 38th day.

Growth and development after definitive dorsal closure

After dorsal closure very little change takes place externally other than the growth of the appendages. Immediately after definitive dorsal closure the abdomen was distended laterally and dorsally in the thoracic and abdominal regions. Segmentation was indistinct and the body wall was translucent. In subsequent days segmentation became more distinctive, the abdomen diminished in bulk, and sclerotization of the integument commenced. Prior to eclosion the chorion became loose and wrinkled due to diminished volume of the termite abdomen. The abdominal region of 38- to

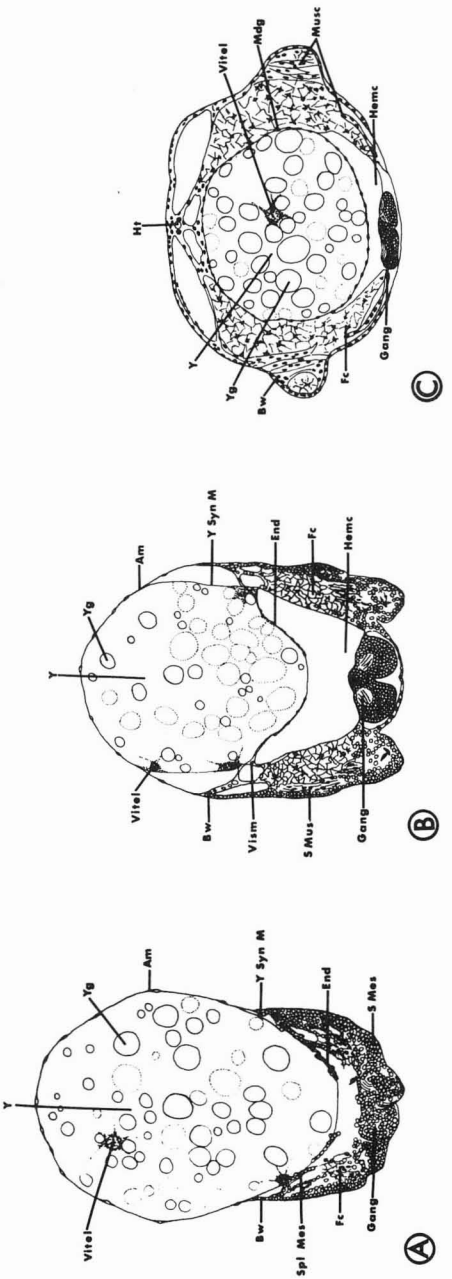

FIG. 9. Stages of dorsal closure in embryo of *C. brevis* during the formation of the definitive body cavity.

46-day-old embryos were generally brown due to yolk in the midgut; in older embryos the diminished yolk appeared as a greenish spot in the abdomen. Removal of the chorion at about the 50th day prompts the embryos to flex their appendages and abdomen. Eclosion generally occurs about the 54th day when the 1st instar termite nymph emerges through the medial suture in the chorion on the posterio-dorsal surface of the egg.

DISCUSSION

Egg characteristics among the termites seem to correlate with taxonomic and phylogenetic groupings. In general, their eggs are curved and about twice as long as they are wide. Of the termites whose embryology has been studied, those belonging to more primitive Kalotermitidae and Hodotermitidae have larger eggs than do those of the highly-evolved Termitidae.

The mean incubation period of *C. brevis* eggs in this study was 56 days at 28 C while in earlier studies (McMahan, 1962) indicated 75 to 81 days at 26 C. The discrepancy may be due to the fact that latter values were estimates obtained in the course of other studies and were not records of individual eggs. *K. flavicollis* similarly has a 54-day incubation period at 26 C. In contrast, eggs of the hodotermitid, *Z. nevadensis,* hatched after 29 days at 26 C. The incubation periods of *E. ripperti* and *O. redemanni* eggs were not determined.

C. brevis eggs, like those of other termites and insects, are yolk-rich and centrolecithal. Their bilateral symmetry, rounded ends, convex dorsal and slightly concave ventral surfaces make them most similar to eggs of *K. flavicollis.*

The internal content of the insect egg is divisible into two parts, the protoplasm and deutoplasm. In *C. brevis* eggs, as in *Eutermes* eggs, only a dispersed protoplasmic reticulum was present in the deutoplasm and no periplasm was observable. This is consistent with the trend among eggs of the more primitive orders in showing a decreasing thickness of periplasm in some and in being entirely absent in others (Agrell, 1964).

The period between syngamy and blastogenesis is generally referred to as "cleavage". The process in *C. brevis* eggs began in the presumptive head region in the posterior pole, as it did in *K. flavicollis* eggs (Striebel, 1960). In contrast, the first cleavage division in *E. ripperti,* occurred near the middle of the yolk mass (Knower, 1900).

The major direction of nuclei migration during cleavage in the eggs of *C. brevis* was towards the anterior pole. In *Eutermes* eggs a proliferation towards the future embryonic area in the posterior pole was also observed, while in *Odontotermes* the nuclei appeared to spread uniformly throughout the yolk.

Cleavage in *C. brevis* appeared to become asynchronous at about the fourth or fifth division. This was indicated by two observations: 1) Nuclei in every stage of mitosis were observed during the fifth cleavage division; if the divisions were still synchronous, nuclei in the same mitotic stage would be expected. 2) Additionally, synchronous divisions should lead to an increase in the number of nuclei according to a geometric progression.

In *C. brevis* eggs odd numbers of nuclei that did not fit this progression were observed at about the 4th division. Asynchrony after the 4th cleavage division was reported for *E. ripperti.* Counce (1961) states that important and fundamental changes, one of which is the loss of synchrony in cleavage division, occur in the insect egg at or about cleavage 6.

In *C. brevis* and other species of termites previously studied, an increase of cells in the posterior pole and a concomitant decrease in other areas occurred prior to differentiation of the embryonic anlage. Some of this increase in nuclear density at the posterior pole was no doubt due to active proliferation. Cells of *C. brevis* eggs were observed undergoing mitosis in this region. In *O. redemanni* eggs, active proliferation of cells was deduced to be the primary cause of increase. Decrease in numbers in the anterior regions of the blastoderm was believed to be due to submergence of cells into the yolk and their subsequent disintegration. Active proliferation and migration of blastoderm cells from the anterior region into the germ area occurred in *E. ripperti.* The situation in *C. brevis* appeared to be similar, for in addition to dividing surface cells in that region there were some cells just below the blastoderm layer. It is unlikely that these submerged cells originated at the surface through mitosis because all division of surface cells were observed in a plane parallel to the surface of the egg. Agrell (1964) states that "In more primitive groups the mitotic activity will be higher in the germ anlage (Seidel, 1929), and in the absence of a periplasmic layer the nuclei with their plasma islands have free mobility and also move actively into the germ area."

The embryo of *C. brevis* possessed characteristics common to all termites. The embryonic disc was many layers thick from the beginning (Strindberg, 1913), the anlage of the amnion was multi-layered, and the serosa had a double mode of origin. The amnio-serosal fold (so designated because it formed the amnion and contributed to the serosa) developed as a medial outgrowth of the upturned posterior edge of the embryonic disc. This fold eventually grew anteriorly over the amniotic depression and formed the amniotic cavity. The outermost layer of cells of the amnio-serosal fold was continuous with the extra-embryonic serosa and later appeared to delaminate from the amnion. The serosa, therefore, was formed from both embryonic tissue and extra-embryonic blastoderm. More commonly among the insects, the amnion and serosa develop from extra-embryonic blastoderm.

The endomesoderm of termites, including *C. brevis,* is formed in a manner common to many primitive orders. That is, grastrular invagination appears to be absent, and at irregular points in the embryonic disc cells are pushed inward towards the yolk and others are separated towards the inner surface from all points by tangential division. Additionally, in *O. redemanni* (Mukerji and Chowdhuri, 1960) cells from both the amniotic fold and germinal epithelium (disc) multiply, detach, and migrate to reinforce the inner layer along the entire length of the embryo. A similar situation seems to exist in *C. brevis.* Striebel (1960) reports that in *Z. nevadensis* and *K. flavicollis* there is an "... epibolic formation of the inner layer".

Endomesoderm in *E. ripperti* is reported to form before the cells of the embryonic disc congregate. With *O. redemanni, K. flavicollis, Z. nevadensis,* and *C. brevis* the inner layer forms after differentiation of the disc but before or concurrently with the appearance of the amnio-serosal fold.

In *C. brevis* eggs, as in other similar hemimetabolous insects (Counce, 1961), the initial embryonic portion of the egg was small relative to the extra-embryonic regions and consisted almost entirely of the presumptive protocephalic region. Additionally, in the eggs of all termites studied to date (including *C. brevis)* the embryonic rudiment always develops on the convex surface just above the curvature of the posterior pole of the egg.

The embryonic head region of termites are always on the convex surface. Consequently, elongation proceeds around the posterior curvature to the opposite flat or concave surface and then towards the anterior pole. This is the pattern of growth regardless of whether surfaces are designated dorsal or ventral.

The trunk region of the termite embryos are formed by elongation and some widening. Elongation is generally accompanied by segmentation.

In *C. brevis,* elongation commenced with growth of the posterior perimeter of the embryonic vesicle on about the 10th day. The original embryonic region was incorporated in the cephalic region. The germ band elongated around the curvature of the posterior egg pole to the ventral surface and then toward the anterior pole. Segmentation began with the metamerization of the mesoderm prior to the 14th day of embryonic life. Externally, segments became apparent on about the 16th day with the sudden appearance of cephalic, gnathal, and anterior thoracic segments. The sudden appearance of these segments was also noted in the embryo of *Eutermes.*

Studies of *K. flavicollis* and *Z. nevadensis* indicated segmentation proceeds from the preantennal segments backward, thus distinguishing these species from Orthoptera, where segmentation starts either in the gnathal or thoracic segments. Abdominal segments formed serially and first appeared in *C. brevis* at the 16th or 17th day; six or seven abdominal segments were present by the 18th day. The segments seemed to differen-

tiate from the anterior margin of the cauda and include the telson, which is identifiable by the proctodael invagination. Such tail pieces are common in embryos of hemimetabolous insects and are called the "segment-forming zone" (Counce, 1961). Segmentation was complete in the abdomen of *C. brevis* and other termite species when there were 10 segments, plus the much-reduced telson, on about the 22nd day. Each abdominal segment possessed a pair of lateral evanescent appendages (pleuropodia) which were well developed in anterior segments but diminished in size caudally. Pleuropodia are believed to serve a variety of functions, including secretion of an enzyme which helps dissolve parts of the egg shell before hatching (Imms, 1960).

Differences among termite embryos in the extent of cephalic flexure and pre-rotational positions appear to exist. Cephalic flexing was absent in *K. flavicollis* and the embryo was ventrally situated. The embryo of *C. brevis* was not totally ventrally situated and the head was flexed to a vertical position. By comparison, *Zootermopsis* showed a more acute flexure with the head section arched over the posterior pole. The two termitids, *E. ripperti* and *O. redemanni,* showed the severest cephalic flexure as the head regions were arched back almost flat against the dorsal surface of the embryo. Caudal flexure was exhibited by all species.

Blastokinesis refers to all displacements and rotations within the egg (Johannsen and Butt, 1941) and is just one kind of morphogenetic movement exhibited by the insect embryo. Blastokinetic movements are believed to be most pronounced in the eggs of hemimetabolous orders such as Orthoptera and Isoptera because they have very short germ bands and, consequently, more room for movement. This trend appears to be present in the order Isoptera, as the larger eggs of the more primitive termites underwent more extensive blastokinetic movements than did the smaller eggs of advanced species. Primitive kalotermitid species exhibited two blastokinetic movements: the first, a ventral displacement, was not observed with the other species; the second, a rotation, was described for all termite species studied to date.

Ventral displacement of the two kalotermitid embryos occurred at different times. The *K. flavicollis* embryo was displaced while still a small globular vesicle, while with *C. brevis* displacement occurred during growth and elongation of the embryo. In both species the embryo moved from the convex to the concave surface.

Rotation occurs in the hodotermitid and two kalotermitids at about the middle of the incubation periods. In these species rotation occurs around two axes, while the termitid embryos rotate around one axis.

There are two phases in the rotational process of *Kalotermes* and *Zootermopsis* and each phase corresponds to rotation around different axes.

The first phase (Striebel, 1960) corresponds to the unrolling of the invaginated or immersed germ in other insect groups. The embryo slides headfirst from the concave ventral surface, around the posterior pole to the convex dorsal surface of the egg, and continues anteriorly until completely dorsal in position. In the second phase, the embryo slides sideways around the yolk and rotates 180 degrees around the longitudinal axis of the egg to the concave ventral side. The embryo thus attains its definitive position on the same ventral side from which rotation commenced, but now with its head and cauda in the anterior and posterior poles of the egg, respectively.

The rotation of *C. brevis* also was around two axes but it was not divided into distinct phases as in *K. flavicollis* and *Z. nevadensis*. Instead, rotation around the longitudinal egg axis commenced soon after the unrolling phase started. Thereafter the embryo proceeded to rotate around both axes simultaneously, and progressed ventro-anteriorly around the yolk until completely ventral in position. As with *K. flavicollis* and *Z. nevadensis*, the embryo's definitive position was on the same concave ventral side but with its head and tail occupying poles opposite to those from which they started.

In contrast, the rotation of the two termitid embryos was around a single axis and corresponded to the unrolling phase. The embryo moves from the concave or flat dorsal surface around the posterior curvature of the egg to the convex ventral side. The arrival of the embryonic head at the anterior pole of the egg marks the end of rotation.

One feature is common to the rotational processes of termites studied to date. The initial and in the case of the termitids embryos the only movement, is from the concave or flat surface to the convex surface via the posterior pole. According to the phylogeny of Isoptera as presented by Weesner (1960), it appears the more primitive kalotermitid and hodotermitid embryos exhibit the two-phased rotation while the advanced termitid embryos possess a single-phased rotation. Whether this difference is of evolutionary significance is not ascertainable at this time. However, if the second phase of rotation was eliminated during the evolution of the higher termites, termitid-like eggs will have resulted because the surfaces are designated ventral, dorsal, etc., according to the final position of the embryo in the egg. Such an egg will thus be ventrally convex, dorsally concave or flat, and the embryonic disc will form on the ventral side near the posterior pole. This egg will be characterized by an embryo showing a rotation only around the left-right axis of the egg and its definitive ventral side on the convex surface.

The secondary dorsal organ is the contracted serosa subsequent to rotation and is well developed in termite embryos. In *C. brevis* it formed and degenerated in the following manner: Rotation commenced with the development of an opening in an area where the amnion and serosa were

fused. A consequence of the formation of the aperture was that the embryo became topologically continuous with the envelopes and no longer was within them. As the embryo rotated out of the envelopes, the serosa containing the yolk was left in the anterior portion of the egg. The yolk gradually was displaced into the amnion and the serosa appeared to contract. During contraction, cells of the serosal epithelium took on a columnar shape and aligned themselves parallel to each other. As the secondary dorsal organ contracted further the lumen diminished in size and displaced the yolk, leaving a compact structure composed only of columnar cells. These cells subsequently degenerated, and the secondary dorsal organ decreased in size until it was overgrown by the body wall and disintegrated internally into the yolk.

Striebel (1960) attributed the initiation of the second phase of rotation in *K. flavicollis* to strong contractions by the posterior part of the secondary dorsal organ. This region of the secondary dorsal organ at a comparable stage in *C. brevis* was the densely nucleated band of columnar cells. This organ may therefore have a vital function in embryological development of termites.

The mesoderm of the *C. brevis* embryo proliferated with the ectoderm during elongation as an underlying monolayer of cells. Well-developed coelomic sacs were present in most of the post-cephalic segments of this species. The mesoderm of the lateral and ventro-medial walls of the coelomic sacs differentiated into somatic musculature. Fat tissue developed by proliferation of the medial wall, while musculature of the viscera formed from the dorsal wall. The definitive epineural sinus developed when the medial mesoderm strand disintegrated and the yolk receded from the region above the nerve cord. The endoderm appeared after provisional dorsal closure by the amnion as a pair of thin ventro-lateral ribbons of cells between the stomodaeum and proctodaeum. The ribbons widened and grew dorsally around the yolk to form the midgut. Dorsal closure was completed by the growth of the body wall around the remaining yolk. The cardioblasts met dorsally and formed the tubular heart at or just before dorsal closure.

The inner layer of *Eutermes rotundiceps* was distinguishable as an outer mesoderm and an inner endoderm (Strindberg, 1913). In *C. brevis* only the one-cell-thick mesoderm was visible; in *E. rotundiceps* the yolk cells formed a layer on the inner side of the endoderm midgut wall and were retained throughout embryonic life. Disintegration of yolk cells was indicated in *C. brevis.*

CONCLUSION

In *C. brevis* and all other termite embryos studied to date syngamy occurred either in the posterior region of the egg or near its midpoint.

Cleavage was superficial and at least in some species became asynchronous early.

The embryonic disc of *C. brevis* and other termites formed on the convex surface of the egg just above the curvature of the posterior pole. Growth was always around the posterior pole to the concave or flat surface.

The young embryos of the kalotermitids exhibited a ventral displacement not found in the eggs of the other species. In all cases, the inner layer formed through the sinking-in of some embryonic disc cells and through proliferation by tangential division. Segmentation proceeded from the head region caudally. A difference exists in the pre-rotational positions of termite embryos. Because of their ventral displacement at an earlier stage, embryos of Kalotermitidae were in a completely or nearly completely ventral position while those of the other families were curved around the posterior pole. The embryonic rotation processes of the kalotermitids and the hodotermitid were around two axes, the left-right and the longitudinal. In contrast, rotation of termitid embryos occurred around one axis and could have resulted from a loss during evolution of the second phase of rotation around the longitudinal axis.

The secondary dorsal organs of the termites are well developed and may play an important role in their embryogenesis. Differentiation of the mesoderm in Isoptera appeared to occur in a manner similar to that of Orthoptera. The endoderm became apparent after rotation and formed the midgut. Definitive dorsal closure occurred by the upward growth and enclosure of the yolk by the body wall.

A comparative examination of termite embryology based on the few species studied to date indicates differences that correlate well with the taxonomy and phylogeny of Isoptera.

The Kalotermitidae, *K. flavicollis* and *C. brevis,* are characterized by ventral displacement of the young embryo and a consequent completely or nearly completely ventral position prior to rotation. Rotation occurs around two axes. The Hodotermitidae, represented by *Z. nevadensis,* show no ventral displacement of the embryo and a pre-rotational position with its head curved around the posterior pole. The rotation of *Z. nevadensis* is around two axes. The termitids *Odontotermes redemanni* and *Eutermes ripperti* are similar to the hodotermitid in having no ventral displacement of the embryo but differ because rotation occurs only around one axis. Thus it appears that during the evolution of the higher termites the ventral displacement of the young embryo and the revolution around the longitudinal axis of the egg may have been lost.

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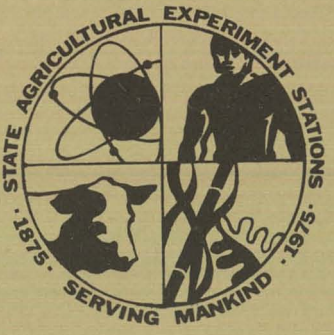

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